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Evaluation of the Effect of Combining *Fusarium oxysporum* and *Macrophomina phaseolina* on the Incidence of Jute (*Corchorus olitorius*) Disease

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ABSTRACT

Background: Jute charcoal rot and wilting caused by *Macrophomina phaseolina* and *Fusarium oxysporum* fungi severely hampered production.

Methods: To determine the combined effects and the interaction of *Macrophomina phaseolina* and *Fusarium oxysporum*, experiments using the double culture technique, inoculation experiments using cut stems and experiments using soil inoculation were designed.

Result: In the experiment with dual cultures, *M. phaseolina* did not exhibit any aggressive behaviour toward *F. oxysporum* and provide results that any abnormality in hyphae of both the fungi. Healthy sesame stems were inoculated with Macrophomina, Fusarium and Macrophomina + Fusarium and it was discovered that the stem colour varied from white to grey to black at different days after inoculation, but the colour of the control stem remained green"during the whole trial period. According to a study on soil inoculation, seed germination rates for inoculations of Macrophomina + Fusarium were as low as 20.00% owing to disease incidence, whereas seed germination rates for inoculations of Macrophomina alone were 30%. The germination rate in the control pot was as high as 78%. Although Macrophomina grows more quickly than Fusarium does, their combination had no antagonistic effects and was shown to make the illness worse than any one of them acting alone.

Key words: Combining effect, *Fusarium oxysporum,* Jute, *Macrophomina phaseolina.*

INTRODUCTION

Soil is a complex ecosystem that serves as a home for a diverse range of creatures that coexist with dozens of other species. Due to their diverse life histories, capacity for dispersion and tolerance to heat, microbiomes in natural settings react to changing climatic circumstances in distinct ways (Khamari *et al.,* 2022). Soil microbial biomass is decreased by rising temperatures and cloud cover (Khamari *et al*., 2022; Rinnan, *et al*., 2006). Precipitation variations influence the makeup of the microbial community (Castro *et al*., 2010). Root-associated bacteria have a significant role in determining the plant community, variety and productivity in the soil (Wardle *et al*., 2004; Wagner *et al*., 2014). As microorganisms are closely associated with plant roots (Bais *et al*., 2006), root phenology plays a significant role in rhizosphere interactions and may affect seasonal assemblages of soil microbial groups.

Many economically significant diseases, including root rot, seedling damping-off and vascular wilts, are caused by soil-borne fungal phytopathogens (Lichtenzveig *et al*., 2006). Jute production is seriously hampered by root rot and wilt diseases brought on by Fusarium species and *Macrophomina phaseolina*, which lower yields and worsen fibre quality. To determine if Macrophomina and Fusarium work together or compete in the Odisha environment, research has been designed.

MATERIALS AND METHODS

Samples for phytopathogenic fungus isolation were gathered from the INS Farm SOA University's jute field. In sterile plastic bags, suspect stems and roots that seemed to have Fusarium wilt and Charcoal Rot symptoms were collected. The removed plant components from the gathered plant samples, such as twigs, bark, stems and roots, were cleaned with sterile water before being surface sterilised with 1% sodium hypochlorite. After that, samples of the roots and stems were divided into 1 cm-long pieces. The researchers used a 7-day-old fresh culture that had been incubated on PDA with 50 mg/L of streptomycin sulphate for 3 to 7 days after being sterilised (Khamari *et al.,* 2019).

In vitro investigation of macrophomina and fusarium's interactions

A dual culture approach was used to explore the interaction between Macrophomina and Fusarium, retaining a 5 mm mycelia disc of each pathogen on the opposite side of the

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petriplates in three replications. A collection of distinct test fungi was kept as a control. Every day, observations were made to examine how they interacted with one another. A tiny piece of mycelia from the area of contact was placed on a slide and it was examined under a microscope.

In vitro investigation of disease complexes

An *in vitro* experiment was carried out to investigate the individual and combined effects of Macrophomina and Fusarium on jute stem. 250 ml of conical flask were filled with 150 ml of potato dextrose broth. Two healthy sesame cut stems, each measuring 10 cm in length and 6.5 mm in diameter, were retained within the flask and were carefully sealed with non-absorbent cotton. 16 conical flasks were also included in the experiment. Every flask was autoclaved.

After chilling, a 5 mm disc of Macrophomina was added to the flask, followed by a second inoculation of Fusarium, a third inoculation of both Macrophomina and Fusarium and a fourth flask that was left uninoculated, serving as the control. The replications were kept at four. After that, these flasks were left alone and routine observations were made at 2, 4, 6 and 14 days following inoculation.

In vivo investigation of disease complexes

The standard protocal recommended by Khamari *et al.* (2019) used to propagate *Macrophomina phaseolina, Fusarium oxysporum* and both, *M. phaseolina + F. oxysporum*, in sand cone media before being injected at a rate of 2 g/kg soil, experimentation were conducted in plastic pots. One treatment was kept uninoculated as a control. All pots were kept in a completely randomized pattern and seeded with J.R.C-212 (Sonali) seed variety. Five replications of each treatment were carried out and observations were made. Disease incidence data were kept daily and during germination and statistical analysis was done to determine how therapy affected certain plant development parameters.

$$DI\ (\%) = \frac{\text{Number of plants affected}}{\text{Total number of plants}} \times 100$$

RESULTS AND DISCUSSION

Dual culture technique

The pathogens multiplied and mixed with one another. Macrophomina and Fusarium did not exhibit any aggressive behaviour against one another. These findings are similar with Khamari *et al*. (2017) and it was also shown that Macrophomina spread faster than Fusarium, covering the majority of the plate. When both diseases were seen together under a microscope, they coexisted.

In vitro cut stem inoculation method

After applying Macrophomina, Fusarium and a combination of both to the sesame stem cuttings, observations were made at 2, 4, 6 and 14 days. There was a thin layer of mycelium covering the medium, which was white in colour. After two days of inoculation, the incidence of Macrophomina and Fusarium was low and the incidence of the two together was moderate. After 4 days following inoculation, the mycelium in the presence of Macrophomina, Fusarium and Macrophomina + Fusarium, respectively, changes to grey, white and white+grey (Table 1).

The mycelium was discovered to have spread up to half the length of the stem together with complete medium coverage by Macrophomina species and only partial medium coverage by Fusarium species without stem infection and a combination of both showed complete moderate coverage with a quarter of stem infection. Due to the inoculation of Macrophomina, the colour gradually changed to a dark grey covering the entire medium and the stem. Contrarily, Fusarium inoculation resulted in the colour turning creamy white and encompassing the entire surface and a section of the stem. Both treatments combined to finally give the colour a greyish tone after six days. After a 14-day inoculation period, we found that Macrophomina, Fusarium and pathogens alone or in combination covered the entire medium and the entire stem, resulting in a change in color to black, milky and greyish. These results are consistent with the study by (Khamari *et al.,* 2017). According to the inoculation research, the stem's colour changed from white to grey to black at various inoculation days when exposed

Table 1: Macrophomina, Fusarium and their combinations' cultural characteristics are evaluated at specified intervals following inoculation.

Treatments	2 DAI	4 DAI	6 DAI	14 DAI
		Mycelium colour		
Control	Green	Green	Dark grey	Black
Macrophomina	White	Grey	Creamy white	Creamy white
Fusarium	White	White	Grey	Grey
Macrophomina+Fusarium	White	White+ grey	Dark grey	Black
Treatments	2 DAI	4 DAI	6 DAI	14 DAI
		Mycelium coverage		
Control	-	-	-	-
Macrophomina	Medium	Medium+half stem	Medium+stem	Medium+stem
Fusarium	Medium	Medium	Medium+1/4th stem	Medium+stem
Macrophomina+Fusarium	Medium	Medium+1/4th stem	Medium+1/2th stem	Medium+stem

Table 2: Assessing the impact of soil inoculation with Macrophomina, Fusarium and their combinations.

Treatments	Germination %	Disease incidence %
Control	78[a]	18[c]
Macrophomina	30[c]	66[ab]
Fusarium	56[b]	54[b]
Macrophomina + *Fusarium*	20[c]	78[a]
LSD (0.05)	16.93	18.10

Means with same letter do not differ significantly (Tukey's HSD).

to Macrophomina, Fusarium and the combination of Macrophomina + Fusarium.

In vivo investigation of disease complexes

The soil inoculation study found that, under controlled conditions, inoculating with Macrophomina and Fusarium resulted in disease incidence rising to 78.00% and 20.00% seed germination, followed by Macrophomina alone (seed germination of 30% and disease incidence of 66%) and Fusarium recording 56% seed germination and 54% disease incidence. The control pots, on the other hand, had 78% of the seeds germinate without any pathogen inoculation. Khamari *et al.* (2017), who carried out the same experiment on sesame, published similar results, which are supported by these findings.

In comparison to Fusarium, Macrophomina activity was shown to be faster in terms of dual culture method and soil inoculation experiment. It has been established that Macrophomina grows more quickly than Fusarium. However, the combination of Macrophomina and Fusarium had no antagonistic effects and it was discovered that both, when present together, made the illness worse than when each was present alone (Table 2).

Numerous researchers have already examined the combined impact of Macrophomina and Fusarium on numerous crops. Noted that *Fusarium verticilloides* and *M. phaseolina* were in charge of collar rot, seedling rot and other infections in okra. Additionally, they noticed that infected seeds resulted in less seed germination and pre- and post-emergence mortality. Brinjal growth is severely reduced by *M. phaseolina* + *Fusarium oxysporum* and carbendazim considerably reduced the fungal complex (Haseeb and Archana, 2009).

CONCLUSION

The study provides significant fresh knowledge about the interaction between *Fusarium oxysporum* and *Macrophomina phaseolina* on the frequency of disease in jute. It further indicates that there are no antagonistic effects between the two funguses. Rather, it was discovered that their combined presence made the infection worse than it would have if either fungus had worked alone.

The results emphasize the deep relationships between plant roots and microorganisms, the intricacy of soil ecosystems and the difficulties in controlling soil-borne fungal phytopathogens, which cause economically important diseases like root rot and wilt in crops like jute. Thus, the study highlights the need for more investigation to gain a deeper understanding of these relationships and create practical plans for managing diseases in jute farming.

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Conflict of interest

Authors declared that they have no competing of interests.

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